

10010390-110504  
FO507T-06207001

**TITLE:**           **METHODS OF INHIBITING DESICCATION OF  
CUTTINGS REMOVED FROM ORNAMENTAL  
PLANTS**

**INVENTORS:**   **Zhong-Min Wei, Ernesto Leon, and Agustin  
Oviedo**

**DOCKET NO.:**   **21829/111 (EBC-009)**

## **METHODS OF INHIBITING DESICCATION OF CUTTINGS REMOVED FROM ORNAMENTAL PLANTS**

This application claims benefit of U.S. Provisional Patent Application  
5 Serial No. 60/248,169, filed November 13, 2000, which is hereby incorporated by  
reference in its entirety.

### **FIELD OF THE INVENTION**

10 The present invention generally relates to methods of treating  
ornamental plants or cuttings removed therefrom to inhibit desiccation of cuttings  
removed from the ornamental plants.

### **BACKGROUND OF THE INVENTION**

15 According to an April 2001 report by the United States Department of  
Agriculture, National Agricultural Statistics Service, Sp Cr 6-1 (01), entitled  
"Floriculture Crops: 2000 Summary", during the previous year the wholesale value of  
domestically produced cut flowers was \$427 million. The top three valued cut flower  
20 categories were Roses at \$69.4 million, Lilies at \$58.6 million, and Gladioli at \$32.2  
million. While the U.S. cut flower industry is not insignificant, two-thirds of the cut  
flowers sold in the U.S. in 1998 were imported, and this import market was worth \$1  
billion. Of the imports coming into the U.S. that year, 56% were from Colombia,  
22% from elsewhere in Central & South America, and about 18% from The  
25 Netherlands.

Postharvest handling methods that were developed over 20 years ago  
on U.S. produced flowers are still current practice in the fresh flower industry.  
However, as noted above, many flowers sold in the U.S. today are imported from  
Colombia and Ecuador and can be 8-10 days old when purchased by consumers.  
30 Current problems with cut flower longevity and quality are associated with shifts in  
the geographical locations of production, introduction of new varieties, long-distance  
transport from farm to consumer, improper transport and storage temperatures, and  
undesirable handling practices. With respect to transport and storage temperatures,

prevalent problems include: flowers are often not pre-cooled adequately when they leave the grower; use of non-refrigerated trucks during shipment; boxed flowers which sit for extended periods on non-refrigerated docks; and flowers are not kept cool during air transport.

5           The effect that these problems can have on cut flower longevity includes not only poor appearance of flowers at retail sites, but also loss of flowers (i.e., wilting or dying) prior to the time they reach the retailer or shortly thereafter. In either case, the wholesaler or the retailer may realize financial losses as a result.

A number of strategies have been devised to minimize flower loss.

10       These include treatment with silver thiosulfate, 1-methylcyclopropene (MCP), carboxymethoxylamine (also known as aminooxyacetic acid (AOAA)), AVG, N-AVG, rhizobitoxine, or L-trans-2-amino-4-methoxy-3-butenoic acid (MVG). Silver thiosulfate and MCP are believed to inhibit the effect of either internal or external ethylene, while the others are believed to act internally to inhibit the ability of the cut  
15       flowers, plants, and fruit to produce ethylene. These compounds (except MCP) are typically applied to plants or plant materials in the form of an aqueous treatment solution. Applications of the treatment solution to potted plants are carried out by spraying it onto the aerial parts of the plants or by including it in the irrigation water which is supplied to their roots. Treatment of cut flowers or greens is typically carried  
20       out by immersing the cut ends of the stems in the aqueous solution containing the treating agent immediately after harvest, during transportation or while the floral arrangement is on display, although they might be treated by immersing the whole flowers into a solution or by spraying them. Since MCP is a gas, it cannot readily be applied in aqueous solution, so plants are treated by exposing them to a modified,  
25       controlled atmosphere (containing a defined amount of MCP) in an enclosed chamber.

Silver thiosulfate is expensive and it may be toxic to animals.

Although MCP is now commercially available, its use is limited due to difficulties in application and its lack of stability.

30       However effective these earlier attempts to reduce cut flower losses, there still exists a need to provide improved, non-toxic and easily practiced approaches for minimizing the losses of ornamental plant cuttings. The present invention is directed to overcoming these deficiencies in the art.

100039-1001

## SUMMARY OF THE INVENTION

5 A first aspect of the present invention relates to a method of inhibiting desiccation of cuttings from ornamental plants which includes: treating an ornamental plant with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit desiccation of a cutting from the ornamental plant after the cutting is removed from the ornamental plant.

10 A second aspect of the present invention relates to a cutting which has been removed from an ornamental plant treated with a hypersensitive response elicitor protein or polypeptide, wherein the cutting is characterized by greater resistance to desiccation as compared to a cutting removed from an untreated ornamental plant.

15 A third aspect of the present invention relates to a method of promoting early flowering of an ornamental plant which includes: treating an ornamental plant with a hypersensitive response elicitor protein or polypeptide under conditions effective to promote early flowering of the ornamental plant.

20 A fourth aspect of the present invention relates to a method of harvesting a cutting from an ornamental plant which includes: treating an ornamental plant with a hypersensitive response elicitor protein or polypeptide and harvesting a cutting from the treated ornamental plant.

25 A fifth aspect of the present invention relates to a method of harvesting a cutting from an ornamental plant which includes: harvesting a cutting from an ornamental plant and treating the harvested cutting with a hypersensitive response elicitor protein or polypeptide.

30 A sixth aspect of the present invention relates to a method of inhibiting desiccation of cuttings from ornamental plants which includes: removing a cutting from an ornamental plant and treating the removed cutting with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit desiccation of the removed cutting.

35 A seventh aspect of the present invention relates to a cutting which has been removed from an ornamental plant, wherein the cutting has been treated with a hypersensitive response elicitor protein or polypeptide and wherein the cutting is characterized by greater resistance to desiccation as compared to an untreated cutting removed from the ornamental plant.

An eight aspect of the present invention relates to a method of inhibiting desiccation of cuttings from ornamental plants which includes: providing a transgenic ornamental plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic ornamental plant or transgenic ornamental plant produced from the transgenic ornamental plant seed under conditions effective to inhibit desiccation in a cutting removed from the transgenic plant.

A ninth aspect of the present invention relates to a method of promoting early flowering of an ornamental plant which includes: providing a transgenic ornamental plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic ornamental plant or transgenic ornamental plant produced from the transgenic ornamental plant seed under conditions effective to promote early flowering of the transgenic ornamental plant.

A tenth aspect of the present invention relates to a method of harvesting a cutting from an ornamental plant which includes: providing a transgenic ornamental plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein; growing the transgenic ornamental plant or transgenic ornamental plant produced from the transgenic ornamental plant seed under conditions; and harvesting a cutting from the grown transgenic ornamental plant, wherein the cutting exhibits a reduced susceptibility to desiccation as compared to cuttings removed from non-transgenic ornamental plants.

An eleventh aspect of the present invention relates to a cutting which has been removed from a transgenic ornamental plant which expresses a heterologous hypersensitive response elicitor protein or polypeptide, wherein the cutting is characterized by greater resistance to desiccation as compared to a cutting removed from a non-transgenic ornamental plant.

A twelfth aspect of the present invention relates to a method of enhancing the longevity of flower blooms on ornamental plant cuttings which includes: providing a transgenic ornamental plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic ornamental plant or transgenic ornamental plant produced

from the transgenic ornamental plant seed under conditions effective to enhancing the longevity of flower blooms on cuttings removed therefrom.

A thirteenth aspect of the present invention relates to a method of enhancing the longevity of flower blooms on ornamental plant cuttings which  
5 includes: treating an ornamental plant with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhancing the longevity of flower blooms on cuttings removed therefrom.

A fourteenth aspect of the present invention relates to a method of enhancing the longevity of flower blooms on ornamental plant cuttings which  
10 includes: harvesting a cutting from an ornamental plant and treating the harvested cutting with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhancing the longevity of flower blooms on the harvested cutting.

Because hypersensitive response elicitor proteins or polypeptides can easily be expressed transgenically in or applied topically to ornamental plants and/or  
15 ornamental plant cuttings, the present invention offers an effective, simple-to-use, non-toxic approach for inhibiting the desiccation of cuttings removed from ornamental plants, promoting early flowering of the ornamental plants, and enhancing the longevity of flower blooms on ornamental plant cuttings. By inhibiting  
20 desiccation of cuttings after they have been removed from an ornamental plant, the cuttings are less likely to wilt and die before they are received by the retailer. This will dramatically decrease losses associated with long transportation rates in less than ideal conditions. Moreover, it is also possible to enhancing the longevity of flower blooms, which end consumers can clearly appreciate.

## 25 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an image illustrating the response of *Vega* roses to pre- and postharvest application of EBC-151 (left), untreated (center), and preharvest only treatment with EBC-151. Image captured 16 days after harvest and postharvest  
30 treatment with EBC-151.

Figure 2 is an image illustrating the response of *Vega* roses to pre-harvest only applications of EBC-151; 150 + 350 g/Ha (left), untreated (center), and

250 g/Ha (right). Image captured 16 days after harvest; no postharvest treatment applied.

Figure 3 is an image illustrating the response of *Vega* roses to postharvest only application of EBC-151. Image captured 16 days after harvest.

5

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of inhibiting desiccation of cuttings from ornamental plants, methods of harvesting cuttings from ornamental  
10 plants, methods of promoting early flowering of ornamental plants, and methods of enhancing the longevity of flower blooms on ornamental plant cuttings.

The ornamental plants can be transgenic plants which express a heterologous hypersensitive response elicitor protein or polypeptide or the ornamental plants can be treated (i.e., via topical application) with a hypersensitive response  
15 elicitor protein or polypeptide. Alternatively, the cutting from the ornamental plant (whether transgenic or not) can itself be treated with a hypersensitive response elicitor protein or polypeptide, independent of any treatment provided to the ornamental plant from which the cutting is removed.

For use in accordance with these methods, suitable hypersensitive  
20 response elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors derived from *Erwinia* species (e.g., *Erwinia amylovora*, *Erwinia*  
25 *chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae*), *Ralstonia* species (e.g., *Ralstonia solanacearum*), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors derived from Gram-positive bacteria. One example is the hypersensitive response  
30 elicitor derived from *Clavibacter michiganensis* subsp. *sepedonicus*.

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitors) from various *Phytophthora* species (e.g., *Phytophthora parasitica*,

*Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*,  
*Phytophthora megasperma*, *Phytophthora citrophthora*, etc.).

Preferably, the hypersensitive response elicitor protein or polypeptide  
is derived from *Erwinia chrysanthemi*, *Erwinia amylovora*, *Pseudomonas syringae*,  
5 *Ralstonia solanacearum*, or *Xanthomonas campestris*.

A hypersensitive response elicitor protein or polypeptide from *Erwinia  
chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as  
follows:

10	Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser	1	5	10	15
	Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser	20	25	30	
15	Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr	35	40	45	
	Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu	50	55	60	
20	Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser	65	70	75	
	Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys	85	90	95	
	Ser	Gly	Gly	Asp	Ala	Leu	Ser	Lys	Met	Phe	Asp	Lys	Ala	Leu	Asp	Asp	100	105	110	
25	Leu	Leu	Gly	His	Asp	Thr	Val	Thr	Lys	Leu	Thr	Asn	Gln	Ser	Asn	Gln	115	120	125	
	Leu	Ala	Asn	Ser	Met	Leu	Asn	Ala	Ser	Gln	Met	Thr	Gln	Gly	Asn	Met	130	135	140	
30	Asn	Ala	Phe	Gly	Ser	Gly	Val	Asn	Asn	Ala	Leu	Ser	Ser	Ile	Leu	Gly	145	150	155	
	Asn	Gly	Leu	Gly	Gln	Ser	Met	Ser	Gly	Phe	Ser	Gln	Pro	Ser	Leu	Gly	165	170	175	
	Ala	Gly	Gly	Leu	Gln	Gly	Leu	Ser	Gly	Ala	Gly	Ala	Phe	Asn	Gln	Leu	180	185	190	
35	Gly	Asn	Ala	Ile	Gly	Met	Gly	Val	Gly	Gln	Asn	Ala	Ala	Leu	Ser	Ala	195	200	205	
	Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val	210	215	220	

FOUO = 06E0F00F

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp  
225 230 235 240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp  
245 250 255

5 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys  
260 265 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln  
275 280 285

10 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr  
290 295 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala  
305 310 315 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala  
325 330 335

15 Asn Ala

This hypersensitive response elicitor protein or polypeptide has a  
molecular mass of 34 kDa, is heat stable, has a glycine content of greater than 16%,  
and contains substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive  
response elicitor protein or polypeptide is encoded by a DNA molecule having a  
nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

cgattttacc cgggtgaacg tgctatgacc gacagcatca cgggtattcga caccggttacg 60  
25 gcggtttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgcccga atccggcgctc 120  
gatctggtat ttcagtttg ggacaccggg cgtgaactca tgatgcagat tcagccgggg 180  
cagcaatata ccggcatgtt gcgcacgctg ctgcctcgtc gttatcagca ggcggcagag 240  
tgcatggtg gccatctgtg cctgaacggc agcgatgtat tgatcctctg gtggccgctg 300  
ccgctcggatc ccggcagtta tccgcagggtg atcgaacggt tgtttgaact ggcgggaatg 360  
30 acgttgccgt cgctatccat agcaccgacg gcgcgtccgc agacagggaa cggacgcgcc 420  
cgatcattaa gataaaggcg gcttttttta ttgcaaaacg gtaacggtga ggaaccggtt 480  
caccgtcggc gtcactcagt aacaagtata catcatgatg cctacatcgg gatcggcgctg 540  
ggcatccgtt gcagataact ttgcgaacac ctgacatgaa tgaggaaacg aaattatgca 600  
aattacgatc aaagcgcaca tcggcggtga tttgggcgct tccggtctgg ggctgggtgc 660  
35 tcagggactg aaaggactga attccgcggc ttcacgctg ggttcacgag tggataaact 720  
gagcagcacc atcgataagt tgacctccgc gctgacttcg atgatgtttg gcggcgcgct 780  
ggcgaggggg ctgggcgcca gctcgaaggg gctggggatg agcaatcaac tgggccagtc 840  
tttcggcaat ggcgcgaggt gtgcgagcaa cctgctatcc gtaccgaaat ccggcggcga 900  
tgcggtgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac 960  
40 caagctgact aaccagagca accaactggc taattcaatg ctgaacgcca gccagatgac 1020  
ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gactgtcgt ccattctcgg 1080  
caacgggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggtt 1140

gcagggcctg agcggcgcggt gtgcattcaa ccagttgggt aatgccatcg gcatgggctg 1200  
 ggggcagaat gctgcgtga gtgcgttgag taacgtcagc acccacgtag acggtaacaa 1260  
 ccgccacttt gtagataaag aagatcgcggt catggcgaaa gagatcgggc agtttatgga 1320  
 tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa 1380  
 5 gacggacgac aaatcctggg cttaaagcgt gagtaaaccg gatgatgacg gtatgaccgg 1440  
 cgccagcatg gacaaattcc gtcaggcgat gggatatgatc aaaagcgcggt tggcggtgga 1500  
 taccggcaat accaacctga acctgcgtgg cgcgggcggt gcatcgctgg gtatcgatgc 1560  
 ggctgtcgtc ggcgataaaa tagccaacat gtcgtgggt aagctggcca acgcctgata 1620  
 atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc 1680  
 10 ttattatgcg gtttatgcggt ttacctggac cgggtaataca tcgtcatcga tctggtacaa 1740  
 acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttcctc 1800  
 gtcgctcaga ttgcgcggt gatggggaac gccgggtgga ataatagagaa actcgccggc 1860  
 cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gcccttttag 1920  
 cagatagatt gcggtttcgt aatcaacatg gtaatgcggt tccgcctgtg cgccggccgg 1980  
 15 gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggt agataccgac 2040  
 aaaatagggc agtttttgcg tggatccgt ggggtgttcc ggctgacaa tcttgagttg 2100  
 gtctcgtcatc atctttctcc atctgggcga cctgatcggt t 2141

The above nucleotide and amino acid sequences are disclosed and  
 20 further described in U.S. Patent No. 5,850,015 to Bauer et al. and U.S. Patent No.  
 5,776,889 to Wei et al., each of which is hereby incorporated by reference in its  
 entirety.

A hypersensitive response elicitor protein or polypeptide derived from  
*Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as  
 25 follows:

	Met	Ser	Leu	Asn	Thr	Ser	Gly	Leu	Gly	Ala	Ser	Thr	Met	Gln	Ile	Ser
	1				5					10					15	
30	Ile	Gly	Gly	Ala	Gly	Gly	Asn	Asn	Gly	Leu	Leu	Gly	Thr	Ser	Arg	Gln
				20					25					30		
	Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Gly	Asn
				35				40					45			
	Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met
	50						55					60				
35	Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Gly	Leu	Met	Gly	Gly	Gly	Leu
	65					70					75					80
	Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu
					85				90						95	
40	Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Asn	Thr
				100					105						110	

	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro
			115					120					125			
	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser
		130					135					140				
5	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln
	145					150					155					160
	Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly
					165					170					175	
10	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu
				180					185					190		
	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly
			195					200					205			
	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly
		210					215					220				
15	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu
	225					230					235					240
	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln
					245					250					255	
20	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln
				260					265					270		
	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe
			275					280					285			
	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met
		290					295					300				
25	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro
	305					310					315					320
	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser
					325					330					335	
30	Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn
			340						345					350		
	Lys	Ala	Lys	Gly	Met	Ile	Lys	Arg	Pro	Met	Ala	Gly	Asp	Thr	Gly	Asn
			355					360					365			
	Gly	Asn	Leu	Gln	Ala	Arg	Gly	Ala	Gly	Gly	Ser	Ser	Leu	Gly	Ile	Asp
		370					375					380				
35	Ala	Met	Met	Ala	Gly	Asp	Ala	Ile	Asn	Asn	Met	Ala	Leu	Gly	Lys	Leu
	385					390					395					400
	Gly	Ala	Ala													

10040390-41054

This hypersensitive response elicitor protein or polypeptide has a molecular mass of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor protein or polypeptide has substantially no cysteine. The hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

```

aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa 60
gaggaatacg ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct 120
atcggcggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg 180
ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg 240
gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcggttg tgggctgatg 300
ggcgggtggc taggcggttg cttaggtaat ggcttgggtg gctcaggttg cctgggcgaa 360
ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa 420
ggcgggaaca ataccacttc aacaacaaat tccccgctgg accaggcgct ggggtattaac 480
tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac 540
ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tgggtgatggg 600
caagatggca cccagggcag ttctctgagg ggcaagcagc cgaccgaagg cgagcagaac 660
gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaatgg tctgagccag 720
ctccttggca acgggggact gggaggtggt cagggcggtg atgctggcac gggctctgac 780
ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag 840
ttaggtaacg ccgtgggtac cggtatcggg atgaaaagcg gcattcaggc gctgaatgat 900
atcggtacgc acaggcacag ttcaaccctg tctttcgtca ataaaggcga tcgggcgatg 960
gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttgccaa gccgcagtac 1020
cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc 1080
aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140
atgatcaaaa ggcccatggc ggggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200
ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260
cttggcaagc tgggcgcggc ttaagctt 1288

```

The above nucleotide and amino acid sequences are disclosed are further described in U.S. Patent No. 5,849,868 to Beer et al. and U.S. Patent No. 5,776,889 to Wei et al., each of which is hereby incorporated by reference in its entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

5 Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu  
1 5 10 15  
Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser  
20 25 30  
10 Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala  
35 40 45  
15 Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly  
50 55 60  
Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly  
65 70 75 80  
20 Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro  
85 90 95  
Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu  
100 105 110  
25 Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp  
115 120 125  
Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp  
130 135 140  
30 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala  
145 150 155 160  
35 Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
165 170 175  
Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro  
180 185 190  
40 Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro  
195 200 205  
45 Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
210 215 220  
Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly  
225 230 235 240  
50 Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His  
245 250 255  
Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln  
260 265 270  
55 Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn  
275 280 285

1001039014501

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val  
 290 295 300  
 5 Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala  
 305 310 315 320  
 Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr  
 325 330 335  
 10 Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn  
 340 345 350  
 Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp  
 355 360 365  
 15 Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe  
 370 375 380  
 20 Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser  
 385 390 395 400  
 His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser  
 405 410 415  
 25 Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu  
 420 425 430  
 Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu  
 435 440 445  
 30

This protein or polypeptide is acidic, rich in glycine and serine, and  
 lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors  
 35 of plant metabolism. The protein or polypeptide of the present invention has a  
 predicted molecular mass of ca. 45 kDa. The DNA molecule encoding this  
 hypersensitive response elicitor protein or polypeptide has a nucleotide sequence  
 corresponding to SEQ. ID. No. 6 as follows:

40 atgtcaattc ttacgcttaa caacaatacc tcgtcctcgc cgggtctggt ccagtcgagg 60  
 ggggacaacg ggcttggtgg tcataatgca aattctgcgt tggggcaaca acccatcgat 120  
 cggcaaacca ttgagcaaat ggctcaatta ttggcggaac tgttaaagtc actgctatcg 180  
 ccacaatcag gtaatgcggc aaccggagcc ggtggcaatg accagactac aggagttggt 240  
 aacgctggcg gcctgaacgg acgaaaaggc acagcaggaa ccaactcgca gtctgacagt 300  
 45 cagaacatgc tgagtgaat gggcaacaac gggctggatc aggccatcac gcccgatggc 360  
 caggcgggcg ggcagatcgg cgataatcct ttactgaaag ccatgctgaa gcttattgca 420  
 cgcgatgatg acggccaaaag cgatcagttt ggccaacctg gtacgggcaa caacagtgcc 480  
 tcttccggta cttcttcacg tggcgggtcc ccttttaacg atctatcagg ggggaaggcc 540  
 ccttccggca actccccttc cggcaactac tctcccgta gtaccttctc accccatcc 600  
 50 acgccaacgt cccctacctc accgcttgat ttcccttctt ctccaccaaa agcagccggg 660  
 ggcagcacgc cggtaacgga tcacccctgac cctgttggtg gcgcgggcat cggggccgga 720

aattcgggtgg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc 780  
 gtgaaagcgg gtcaggtggt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta 840  
 ggcgatggcg gccagtctga aaaccagaaa ccgctgttta tactggaaga cggtgccagc 900  
 ctgaaaaaacg tcaccatggg cgacgacggg gcggatggta ttcattctta cggtgatgcc 960  
 5 aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020  
 agcgcgggca aaaaatccca cgttgaaatc actaacagtt ccttcgagca cgcctctgac 1080  
 aagatcctgc agctgaatgc cgataactaac ctgagcggtg acaacgtgaa ggccaaagac 1140  
 tttggtactt ttgtacgcac taacggcggt caacagggtg actgggatct gaatctgagc 1200  
 catatcagcg cagaagacgg taagttctcg ttcgttaaaa gcgatagcga ggggctaaac 1260  
 10 gtcaatacca gtgatatctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320  
 gccaacctga aggtggctga atga 1344

The above nucleotide and amino acid sequences are disclosed and  
 further described in U.S. Patent No. 6,262,018 to Kim et al., which is hereby  
 15 incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from  
*Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 7  
 as follows:

20 Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
 1 5 10 15  
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
 20 25 30  
 25 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
 35 40 45  
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
 50 55 60  
 30 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
 65 70 75 80  
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
 85 90 95  
 35 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
 100 105 110  
 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125  
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140  
 40 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160

1001039-10501

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175  
 Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile  
 180 185 190  
 5 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly  
 195 200 205  
 Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser  
 210 215 220  
 10 Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser  
 225 230 235 240  
 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp  
 245 250 255  
 Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val  
 260 265 270  
 15 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln  
 275 280 285  
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290 295 300  
 20 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305 310 315 320  
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325 330 335  
 Asn Gln Ala Ala Ala  
 340

This hypersensitive response elicitor protein or polypeptide has a  
 molecular mass of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine  
 and tyrosine. Further information about the hypersensitive response elicitor derived  
 from *Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv.  
 30 *syringae* Harpin<sub>PSS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the  
 Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby  
 incorporated by reference in its entirety. The DNA molecule encoding this  
 hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide  
 sequence corresponding to SEQ. ID. No. 8 as follows:

35  
 atgcagagtc tcagtcttaa cagcagctcg ctgcaaacc cggcaatggc ccttgctctg 60  
 gtacgtcctg aagccgagac gactggcagt acgtcgagca aggcgcttca ggaagttgtc 120  
 gtgaagctgg ccgaggaact gatgcgcaat ggtcaactcg acgacagctc gccattggga 180

aaactgttgg ccaagtcgat ggccgcagat ggcaaggcgg gcggcgggtat tgaggatgtc 240  
atcgctgcgc tggacaagct gatccatgaa aagctcgggtg acaacttcgg cgcgtctgcg 300  
gacagcgcct cgggtaccgg acagcaggac ctgatgactc aggtgctcaa tggcctggcc 360  
aagtcgatgc tcgatgatct tctgaccaag caggatggcg ggacaagctt ctccgaagac 420  
5 gatatgccga tgctgaacaa gatcgcgcag ttcattggatg acaatcccgc acagtttccc 480  
aagccggact cgggctcctg ggtgaacgaa ctcaagggaag acaacttcct tgatggcgac 540  
gaaacggctg cgttcctggtt ggactcgcac atcattggcc agcaactggg taatcagcag 600  
agtgcgctg gcagtctggc agggacgggt ggaggtcttg gcactccgag cagtttttcc 660  
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc 720  
10 ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780  
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgacagc cggtagctcg 840  
gcgaatggcg gacagtcgcg tcaggatctt gatcagttgc tgggcggctt gctgctcaag 900  
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960  
gcgcaaatcg ccaccttgct ggtcagtagc ctgctgcaag gcacccgcaa tcaggctgca 1020  
15 gcctga 1026

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,708,139 to Collmer et al. and U.S. Patent No. 5,776,889 to Wei et al., each of which is hereby incorporated by reference in its entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu  
1 5 10 15  
Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly  
20 25 30  
Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly  
35 40 45  
Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val  
50 55 60  
Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile  
65 70 75 80  
Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr  
85 90 95  
Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln  
100 105 110  
Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser  
115 120 125

FOUO = 06E0100T

	Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr	130	135	140
5	Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly	145	150	155
	Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly	165	170	175
10	Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr	180	185	190
	Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr	195	200	205
15	Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile	210	215	220
	Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp	225	230	235
20	Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp	245	250	255
	Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr	260	265	270
	Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val	275	280	285
30	Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln	290	295	300
	Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala	305	310	315
35	Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp	325	330	335
	Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe	340	345	350
	Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln	355	360	365
45	Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly	370	375	380
	Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr	385	390	395
50	Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln	405	410	415
55	Ala Ser Thr Gln His Thr Glu Leu	420		

This protein or polypeptide is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids. The DNA molecule encoding this hypersensitive

response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 10 as follows:

	tccacttcgc	tgattttgaa	attggcagat	tcatagaaac	gttcaggtgt	ggaaatcagg	60
5	ctgagtgcgc	agatttcgtt	gataaggggtg	tggtactggt	cattgttggt	catttcaagg	120
	cctctgagtg	cgggtcggag	caataccagt	cttccctgctg	gcgtgtgcac	actgagtgcg	180
	aggcataggc	atttcagttc	cttgcggttg	ttgggcatat	aaaaaaagga	acttttataaa	240
	acagtgcaat	gagatgccgg	caaaacggga	accggctcgt	gcgctttgcc	actcacttcg	300
	agcaagctca	accccaaaca	tccacatccc	tatcgaacgg	acagcgatac	ggccacttgc	360
10	tctggtaaac	cctggagctg	gcgtcgggtc	aattgcccac	ttagcgaggt	aacgcagcat	420
	gagcatcggc	atcacacccc	ggccgcaaca	gaccaccacg	ccactcgatt	tttcggcgct	480
	aagcggcaag	agtcctcaac	caaacacgtt	cggcgagcag	aacactcagc	aagcgatcga	540
	cccagtgca	ctgttggtcg	gcagcgacac	acagaaagac	gtcaacttcg	gcacgcccga	600
	cagcaccgtc	cagaatccgc	aggacgccag	caagcccaac	gacagccagt	ccaacatcgc	660
15	taaattgatc	agtgeattga	tcatgtcggt	gctgcagatg	ctcaccaact	ccaataaaaa	720
	gcaggacacc	aatcaggaac	agcctgatag	ccaggctcct	ttccagaaca	acggcgggct	780
	cggtaacccg	tcggccgata	gcggggcgcg	cggtaacccg	gatgcgacag	gtggcgggcg	840
	cggtgatacg	ccaagcgcaa	caggcgggtg	cggcgggtgat	actccgaccg	caacaggcgg	900
	tgggcggcagc	ggtggcgggc	gcacacccac	tgcaacaggt	ggcggcagcg	gtggcacacc	960
20	cactgcaaca	ggcgggtggc	aggggtgggt	aacaccgcaa	atcactccgc	agttggccaa	1020
	ccctaaccgt	acctcaggta	ctggctcggt	gtcggacacc	gcaggttcta	ccgagcaagc	1080
	cggcaagatc	aatgtggtga	aagacaccat	caaggtcggc	gctggcgagg	tctttgacgg	1140
	ccacggcgca	accttcactg	ccgacaaatc	tatgggtaac	ggagaccagg	gcgaaaatca	1200
	gaagcccatg	ttcgagctgg	ctgaaggcgc	tacgttgaag	aatgtgaacc	tgggtgagaa	1260
25	cgaggtcgat	ggcatccacg	tgaaagccaa	aaacgctcag	gaagtcacca	ttgacaacgt	1320
	gcatgcccag	aacgtcggtg	aagacctgat	tacgggtcaa	ggcgagggag	gcgcagcggt	1380
	cactaatctg	aacatcaaga	acagcagtgc	caaaggtgca	gacgacaagg	ttgtccagct	1440
	caacgccaac	actcacttga	aaatcgacaa	cttcaaggcc	gacgatttcg	gcacgatggg	1500
	tcgcaccaac	ggtggcaagc	agtttgatga	catgagcatc	gagctgaacg	gcatcgaaag	1560
30	taaccacggc	aagttcgccc	tggtgaaaag	cgacagtgc	gatctgaagc	tggcaacggg	1620
	caacatcgcc	atgaccgacg	tcaaacacgc	ctacgataaa	acccaggcat	cgacccaaca	1680
	caccgagctt	tgaatccaga	caagtagctt	gaaaaaaggg	ggtggactc		1729

35 The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 6,172,184 to Collmer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Ralstonia solanacearum* has an amino acid sequence corresponding to SEQ. ID.

40 No. 11 as follows:

Met	Ser	Val	Gly	Asn	Ile	Gln	Ser	Pro	Ser	Asn	Leu	Pro	Gly	Leu	Gln
1				5				10					15		

	Asn	Leu	Asn	Leu	Asn	Thr	Asn	Thr	Asn	Ser	Gln	Gln	Ser	Gly	Gln	Ser	
				20					25					30			
	Val	Gln	Asp	Leu	Ile	Lys	Gln	Val	Glu	Lys	Asp	Ile	Leu	Asn	Ile	Ile	
			35					40					45				
5	Ala	Ala	Leu	Val	Gln	Lys	Ala	Ala	Gln	Ser	Ala	Gly	Gly	Asn	Thr	Gly	
		50					55					60					
	Asn	Thr	Gly	Asn	Ala	Pro	Ala	Lys	Asp	Gly	Asn	Ala	Asn	Ala	Gly	Ala	
	65					70					75					80	
10	Asn	Asp	Pro	Ser	Lys	Asn	Asp	Pro	Ser	Lys	Ser	Gln	Ala	Pro	Gln	Ser	
					85					90					95		
	Ala	Asn	Lys	Thr	Gly	Asn	Val	Asp	Asp	Ala	Asn	Asn	Gln	Asp	Pro	Met	
				100					105					110			
	Gln	Ala	Leu	Met	Gln	Leu	Leu	Glu	Asp	Leu	Val	Lys	Leu	Leu	Lys	Ala	
			115					120					125				
15	Ala	Leu	His	Met	Gln	Gln	Pro	Gly	Gly	Asn	Asp	Lys	Gly	Asn	Gly	Val	
		130					135					140					
	Gly	Gly	Ala	Asn	Gly	Ala	Lys	Gly	Ala	Gly	Gly	Gln	Gly	Gly	Leu	Ala	
	145					150					155					160	
20	Glu	Ala	Leu	Gln	Glu	Ile	Glu	Gln	Ile	Leu	Ala	Gln	Leu	Gly	Gly	Gly	
					165					170					175		
	Gly	Ala	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Val	Gly	Gly	Ala	Gly	Gly	
				180					185					190			
	Ala	Asp	Gly	Gly	Ser	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Ala	Asn	Gly	Ala	
			195					200					205				
25	Asp	Gly	Gly	Asn	Gly	Val	Asn	Gly	Asn	Gln	Ala	Asn	Gly	Pro	Gln	Asn	
		210					215					220					
	Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Asp	Gly	Ser	Glu	Asp	
	225					230					235					240	
30	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn	
					245					250					255		
	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Gly	Asn	Gln	
				260					265					270			
	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly	
			275					280					285				
35	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser	
		290					295					300					
	Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val	
	305					310					315					320	
40	Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Gly	Ser	Gln	
					325					330					335		

100109-40501

Gln Ser Thr Ser Thr Gln Pro Met  
340

Further information regarding this hypersensitive response elicitor  
5 protein or polypeptide derived from *Ralstonia solanacearum* is set forth in Arlat, M.,  
et al., "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific  
Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*,"  
EMBO J. 13:543-533 (1994), which is hereby incorporated by reference in its  
entirety. It is encoded by a DNA molecule from *Ralstonia solanacearum* having a  
10 nucleotide sequence corresponding SEQ. ID. No. 12 as follows:

atgtcagtcg gaaacatcca gagcccgctc aacctcccgg gtctgcagaa cctgaacctc 60  
aacaccaaca ccaacagcca gcaatcgggc cagtcctgtc aagacctgat caagcaggtc 120  
gagaaggaca tcctcaacat catcgagcc ctcgtgcaga aggccgcaca gtcggcgggc 180  
15 ggcaacaccg gtaacaccgg caacgcggcg gcgaaggacg gcaatgccaa cgcgggcgcc 240  
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc 300  
ggcaacgtcg acgacgcaa caaccaggat ccgatgcaag cgtgatgca gctgctggaa 360  
gacctggtga agctgctgaa ggcgccctg cacatgcagc agcccggcgg caatgacaag 420  
ggcaacggcg tgggcgggtg caacggcgcc aagggtgccg gcggccaggg cggcctggcc 480  
20 gaagcgctgc aggagatcga gcagatcctc gccagctcgc gcggcgggcg tgctggcgcc 540  
ggcgggcgcg gtggcggtgt cggcggtgct ggtggcgcg atggcggtc cggtgcggtg 600  
ggcgcaggcg gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac 660  
ggcccgagca acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac 720  
cagggcggcc tcaccggcgt gctgcaaaaag ctgatgaaga tcctgaacgc gctggtgcag 780  
25 atgatgcagc aaggcggcct cggcgggcgg aaccaggcgc agggcggtc gaagggtgcc 840  
ggcaacgcct cgccggcttc cggcgcgaa cggggcgca accagcccgg ttcggcggat 900  
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc 960  
gtccagatcc tgcagcagat gctggcgggc cagaacggcg gcagccagca gtccacctcg 1020  
acgcagccga tgtaa 1035  
30

The above nucleotide and amino acid sequences are disclosed and  
further described in U.S. Patent No. 5,776,889 to Wei et al., which is hereby  
incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from  
35 *Xanthomonas campestris* has an amino acid sequence corresponding to SEQ. ID.  
No. 13 as follows:

Met Asp Ser Ile Gly Asn Asn Phe Ser Asn Ile Gly Asn Leu Gln Thr  
1 5 10 15

Met Gly Ile Gly Pro Gln Gln His Glu Asp Ser Ser Gln Gln Ser Pro  
20 25 30

5 Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln Leu Leu Ala Met Phe Ile  
35 40 45

Met Met Met Leu Gln Gln Ser Gln Gly Ser Asp Ala Asn Gln Glu Cys  
50 55 60

10 Gly Asn Glu Gln Pro Gln Asn Gly Gln Gln Glu Gly Leu Ser Pro Leu  
65 70 75 80

Thr Gln Met Leu Met Gln Ile Val Met Gln Leu Met Gln Asn Gln Gly  
85 90 95

15 Gly Ala Gly Met Gly Gly Gly Gly Ser Val Asn Ser Ser Leu Gly Gly  
100 105 110

20 Asn Ala

This hypersensitive response elicitor protein has an estimated molecular mass of about 12 kDa based on the deduced amino acid sequence, which is consistent with the molecular mass of about 14 kDa as detected by SDS-PAGE. It is encoded by a DNA molecule from *Xanthomonas campestris* having a nucleotide sequence corresponding SEQ. ID. No. 14 as follows:

atggactcta tcggaaacaa cttttcgaat atcggcaacc tgcagacgat gggcatcggg 60  
cctcagcaac acgaggactc cagccagcag tcgccttcgg ctggctccga gcagcagctg 120  
30 gatcagttgc tcgccatgtt catcatgatg atgctgcaac agagccaggg cagcagtgca 180  
aatcaggagt gtggcaacga acaaccgcag aacggtcaac aggaaggcct gagtccgttg 240  
acgcagatgc tgatgcagat cgtgatgcag ctgatgcaga accagggcgg cgccggcatg 300  
ggcgggtggcg gttcgggtcaa cagcagcctg ggcggcaacg cc 342

The above protein and nucleic acid molecule are further described in U.S. Patent Application Serial No. 09/412,452 to Wei et al., filed April 9, 2001, which is hereby incorporated by reference in its entirety.

Other embodiments of the present invention include, but are not limited to, use of hypersensitive response elicitor proteins or polypeptides derived from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of an *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN*<sub>Ecc</sub> and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves,"

MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996  
5 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, each of which is hereby incorporated by reference in its entirety.

Hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors  
10 from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and  
15 Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet, et al., "Acquired Resistance Triggered by  
20 Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), each of which is hereby incorporated by reference in its entirety.

Another hypersensitive response elicitor protein or polypeptide which can be used in accordance with the present invention is derived from *Clavibacter michiganensis* subsp. *sepedonicus* and is described in U.S. Patent Application Serial  
25 No. 09/136,625 to Beer et al., filed August 19, 1998, which is hereby incorporated by reference in its entirety.

Fragments of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens can also be used according to the present invention.

30 Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory,

Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding editions), each of which is hereby incorporated by reference in its entirety. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference in its entirety.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference in its entirety. These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ.

ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. DNA molecules encoding these fragments can also be utilized in a chimeric gene of the present invention.

10 Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

15 The hypersensitive response elicitor proteins or polypeptides used in accordance with the present invention are preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the hypersensitive response elicitor protein or polypeptide of interest is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC. Alternatively, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells (discussed *infra*) and removed therefrom.

30 One particular hypersensitive response elicitor protein, known as harpin<sub>Ea</sub>, is commercially available from Eden Bioscience Corporation (Bothell, Washington) under the name of Messenger<sup>®</sup>. Messenger<sup>®</sup> contains 3% by weight of harpin<sub>Ea</sub> as the active ingredient and 97% by weight inert ingredients. Harpin<sub>Ea</sub> is one

type of hypersensitive response elicitor protein from *Erwinia amylovora*, identified herein by SEQ. ID. No. 3.

Other hypersensitive response elicitors can be readily identified by isolating putative protein or polypeptide candidates and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art.

DNA molecules encoding other hypersensitive response elicitor proteins or polypeptides can also be identified by determining whether such DNA molecules hybridizes under stringent conditions to a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, 8, 10, 12, or 14. An example of suitable stringency conditions is when hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the temperature for either hybridization or washing conditions or increasing the sodium concentration of the hybridization or wash medium. Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 µg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an

expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference in its entirety), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference in its entirety.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems

infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eukaryotic promoters differ from those of prokaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a prokaryotic system, and, further, prokaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of mRNA in prokaryotes depends upon the presence of the proper prokaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in prokaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference in its entirety.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the  $P_R$  and  $P_L$  promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*,

and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

5               Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc.,  
10               are under different controls.

                  Specific initiation signals are also required for efficient gene transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector,  
15               which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not  
20               limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

                  Once the isolated DNA molecule encoding the hypersensitive response  
25               elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

30               Because it is desirable for recombinant host cells to secrete the hypersensitive response elicitor protein or polypeptide, it is preferable that the host cell also be transformed with a type III secretion system in accordance with Ham et al., "A Cloned *Erwinia chrysanthemi* Hrp (Type III Protein Secretion) System

Functions in *Escherichia coli* to Deliver *Pseudomonas syringae* Avr Signals to Plant Cells and Secrete Avr Proteins in Culture," Microbiol. 95:10206-10211 (1998), which is hereby incorporated by reference in its entirety.

Isolation of the hypersensitive response elicitor protein or polypeptide  
5 from the host cell or growth medium can be carried out as described above.

The methods of the present invention can be performed by treating the ornamental plant or a cutting removed therefrom.

Before removal of a cutting, suitable application methods include, without limitation, high or low pressure spraying of the entire plant. After removal of  
10 a cutting, suitable application methods include, without limitation, low or high pressure spraying, coating, or immersion. Other suitable application procedures (both pre- and post-cutting) can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor protein or polypeptide with the cutting. Once treated, the cuttings can be handled, packed, shipped, and  
15 processed using conventional procedures to deliver the cuttings to distributors or end-consumers.

The hypersensitive response elicitor polypeptide or protein can be applied to cuttings in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or  
20 protein can be applied separately to cuttings with other materials being applied at different times.

A composition suitable for treating ornamental plants or cuttings therefrom in accordance with the application embodiment of the present invention contains an isolated hypersensitive response elicitor polypeptide or protein in a  
25 carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. The composition preferably contains greater than about 500 nM hypersensitive response elicitor polypeptide or protein, although greater or lesser amounts of the hypersensitive response elicitor polypeptide or protein depending on the rate of composition application and efficacy of different hypersensitive response elicitor  
30 proteins or polypeptides.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof.

10010390:410501  
FOUO:062000F

Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and ripening agents. These materials can be used either to facilitate the process of the present invention or to provide additive benefits to inhibit desiccation or promote flowering.

As indicated above, one embodiment of the present invention involves treating ornamental plants or their cuttings with an isolated hypersensitive response elicitor protein or polypeptide. The hypersensitive response elicitor protein or polypeptide can be isolated from its natural source (e.g., *Erwinia amylovora*, *Pseudomonas syringae*, etc.) or from recombinant source transformed with a DNA molecule encoding the protein or polypeptide.

Another aspect of the present invention relates to a DNA construct as well as host cells, expression systems, and transgenic plants which contain the heterologous DNA construct.

The DNA construct includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule and which is effective to transcribe the DNA molecule in the tissues of cuttings, and a 3' regulatory region operably coupled to the DNA molecule. Expression of the DNA molecule in such tissues imparts to a cutting resistance against desiccation.

Expression of such heterologous DNA molecules requires a suitable promoter which is operable in plant tissues. In some embodiments of the present invention, it may be desirable for the heterologous DNA molecule to be expressed in many, if not all, tissues. Such promoters yield constitutive expression of coding sequences under their regulatory control. Exemplary constitutive promoters include, without limitation, the nopaline synthase promoter (Fraley et al., Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 35S promoter (O'Dell et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety). Other constitutive plant promoters are continuously being identified and can be used in accordance with the present invention.

While constitutive expression is generally suitable for expression of the DNA molecule, it should be apparent to those of skill in the art that temporally or tissue regulated expression may also be desirable, in which case any regulated promoter can be selected to achieve the desired expression. Typically, the temporally or tissue regulated promoters will be used in connection with the DNA molecule that are expressed at only certain stages of development or only in certain tissues.

In another embodiment of the present invention, expression of the heterologous DNA molecule is directed in a tissue-specific manner or environmentally-regulated manner (i.e., inducible promoters). Tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues.

Promoters useful for expression in leaf tissue include the Rubisco small subunit promoter.

Promoters useful for expression in flower tissues include the 5-enolpyruvylshikimate-3-phosphate synthase promoter (Benfy, et al., "Sequence Requirements of the 5-enolpyruvylshikimate-3-phosphate Synthase 5'-Upstream Region for Tissue-Specific Expression in Flowers and Seedlings," The Plant Cell 2:849-856 (1990), which is hereby incorporated by reference in its entirety) and the tomato PG  $\beta$ -subunit promoter (U.S. Patent No. 6,127,179 to DellaPenna et al., which is hereby incorporated by reference).

Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, or the presence of light. In some plants, it may also be desirable to use promoters which are responsive to pathogen infiltration or stress. For example, it may be desirable to limit expression of the protein or polypeptide in response to infection by a particular pathogen of the plant. One example of a pathogen-inducible promoter is the *gstI* promoter from potato, which is described in U.S. Patent Nos. 5,750,874 and 5,723,760 to Strittmayer et al., each of which is hereby incorporated by reference in its entirety.

Expression of the DNA molecule in isolated plant cells or tissue or whole plants also utilizes appropriate transcription termination and polyadenylation of mRNA. Any 3' regulatory region suitable for use in plant cells or tissue can be operably linked to the first and second DNA molecules. A number of 3' regulatory

regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the

5 cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

The promoter and a 3' regulatory region can readily be ligated to the

10 DNA molecule using well known molecular cloning techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference in its entirety.

One approach to transforming plant cells with a DNA molecule of the

15 present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., each of which is hereby incorporated by reference in its entirety. Generally, this procedure

20 involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is

25 carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

Another method of introducing the DNA molecule into plant cells is

30 fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the DNA molecule. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference in its entirety.

10010390-14501  
FOUO: 06E01001

5    Electrical impulses of high field strength reversibly permeabilize biomembranes  
allowing the introduction of the plasmids. Electroporated plant protoplasts reform the  
cell wall, divide, and regenerate.

15 *Agrobacterium* is a representative genus of the Gram-negative family  
Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy  
root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are  
induced to produce amino acid derivatives known as opines, which are catabolized  
only by the bacteria. The bacterial genes responsible for expression of opines are a  
20 convenient source of control elements for chimeric expression cassettes. In addition,  
assaying for the presence of opines can be used to identify transformed tissue.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the DNA molecule of

the present invention. Suitable selection markers include, without limitation, markers coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection media containing an antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present invention relates to a transgenic ornamental plant that includes a heterologous DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, wherein the heterologous DNA molecule is under control of a promoter that induces transcription of the DNA molecule in tissues of cuttings. Preferably, the DNA molecule is stably inserted into the genome of the transgenic plant of the present invention.

Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics is hereby incorporated by reference in its entirety.

It is known that practically all plants can be regenerated from cultured cells or tissues, including both monocots and dicots.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If

these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the DNA molecule encoding the hypersensitive response elicitor protein or polypeptide is stably incorporated in transgenic plants, it can be transferred  
5 to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the field.

With respect to desiccation, complete protection against desiccation  
10 may not be conferred, but the severity of desiccation can be reduced. Desiccation protection inevitably will depend, at least to some extent, on other conditions such as storage temperatures, light exposure, etc. However, this method of controlling desiccation has the potential for eliminating some other treatments (i.e., additives to water, thermal regulation, etc.) which may contribute to reduced costs or, at least,  
15 substantially no increase in costs. Moreover, by controlling desiccation, it is also possible to enhance the longevity of flower blooms.

The methods of the present invention can be utilized to treat a wide variety of ornamental plants to control desiccation of cuttings removed therefrom as well as enhance the longevity of flowers. Ornamental plants can be either monocots  
20 or dicots. Cuttings include stems, leaves, flowers, or combinations thereof.

In addition to treatment with hypersensitive response elicitor proteins or polypeptides, as well as transgenic expression thereof in tissues of cuttings, cuttings or ornamental plants (transgenic or otherwise) can also be treated with ethylene action inhibitors of the types disclosed in U.S. Patent No. 6,194,350 to  
25 Sisler, U.S. Patent No. 6,153,559 to Heiman, and U.S. Patent No. 5,518,988 to Sisler et al., each of which is hereby incorporated by reference in its entirety. Such treatment can occur before harvest, after harvest, or both. One commercially available ethylene-action inhibitor is EthylBloc® (1-methylcyclopropene, available from AgroFresh Inc. and Floralife Inc.).

105011-0601001

## EXAMPLES

The following examples are intended to illustrate, but by no means are intended to limit, the scope of the present invention as set forth in the appended  
5 claims.

### **Example 1- Increased Flower Quality and Longevity of Roses from Postharvest Application of EBC-151 (Messenger®)**

Mature rose plants were treated with Messenger® (coded as EBC-151)  
10 by foliar sprays and postharvest treatment to improve flower quality and longevity. The trial was established in a commercial rose greenhouse in Villa Guerrero, Mexico. The rose variety in this trial was *Vega*. Individual plot beds contained approximately 44 mature plants arranged in two rows; each plot was replicated 4 times and measured 80 cm wide by 15.4 m long. EBC-151 treatments were applied with a CO<sub>2</sub>-powered  
15 backpack sprayer calibrated to deliver 430 l/Ha at 90 psi. Treatment rates and timings in this trial are shown in Table 1 below.

Table 1: Application rates and treatment schedule for EBC-151 to *Vega* roses

Treatment	EBC-151 Application Rate	Treatment Details
1	250 g/Ha	8 applications at approximately 14-d intervals
2	250 g/Ha + 3.33 g/L postharvest spray	8 applications at approximately 14-d intervals followed by a postharvest spray to 10 commercially-harvested flower/stems within 1 hour of cutting
3	150 g/Ha + 350 g/Ha	150 g/Ha applied 5 times followed by 350 g/Ha applied 3 times at the same 14-d schedule, no postharvest application
4	150 g/Ha + 350 g/Ha + 3.33 g/L postharvest spray	150 g/Ha applied 5 times followed by 350 g/Ha applied 3 times at the same 14-d schedule followed by a postharvest spray to 10 commercially-harvested flower/stems within 1 hour of cutting
5	3.33 g/L postharvest spray only	Postharvest spray only to 10 commercially-harvested flower/stems within 1 hour of cutting
6	N/a	Untreated with EBC-151

Preharvest applications of each EBC-151 treatment were repeated at  
20 approximately 14-d intervals. After the fifth preharvest application, 10 mature flower/stems were randomly selected from each treatment and evaluated. Treatment effects were evaluated on cut flowers by assessing the number of open flowers and the

number of "straight" stems on each flower/stem. An "open" flower was determined to conform to commercial standards for sale by having flower petals extended. Flower petals judged as partially extended were rated as "not open". Straight stems were evaluated as conforming to commercial standard of acceptability for sale.

- 5 Results for this evaluation are shown in Table 2 below. No postharvest applications of EBC-151 were made to flower/stems harvested after the fifth application of EBC-151.

Table 2: Response of cut *Vega* roses to treatment with EBC-151 (five applications only)

Treatment	Number of Flowers	Number of "Open" Flowers	Percent "open" Flowers	Number of Flowers with "Straight" Stems
1	10	10	100	10
3	10	2	20	6
6	10	1	10	4

- 10 Additional preharvest treatments continued with three more applications (for a total of eight applications). Following the eighth application, an additional 10 mature flower/stems were then randomly selected from each treatment and evaluated in the same manner as had been done after the fifth application. Immediately after cutting (within 1 hour) a single postharvest treatment of EBC-151
- 15 was applied at the rate of 3.33 g/L (100 ppm a.i.) to the cut flower/stems harvest from Treatments 2, 4 and 5. The postharvest spray was applied by completely misting each flower/stem with the EBC-151 solution. Sixteen days after postharvest treatment, the number of open flowers and number of flowers with "straight" stems were determined for each treatment. Results for this evaluation are shown in Table 3 below.

20

Table 3: Response of cut *Vega* roses to treatment with EBC-151 (eight preharvest and one postharvest application)

Treatment	Number of Flowers	Number of "Open" Flowers	Percent "open" Flowers	Number of Flowers with "Straight" Stems
1	10	9	90	8
2	10	10	100	8
3	10	9	90	9
4	10	10	100	9
5	10	3	30	1
6	10	2	20	2

Visual observations of cut roses 16 days after postharvest treatment were made for treatments that received postharvest applications of EBC-151. Roses that had been treated with the postharvest application of EBC-151 appeared to have substantially greater longevity than those that had not received the postharvest treatment (Figures 1-3).

Results of this trial demonstrated a treatment effect for application of EBC-151 (Messenger<sup>®</sup>) to roses. The effect was seen in a substantially greater increase in the number of open flowers at harvest. This effect is of significant commercial benefit to rose growers. In addition, the postharvest application of EBC-151 to cut roses resulted in substantially extending the "shelf life" of the cut roses.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

10010390-110501  
TDSUTT-06201001